IN THE SPECIFICATION

Please enter the SEQUENCE LISTING into the specification.

Please amend the paragraph starting on page 4, line 11 as follows:

The invention provides multiplexing of nonluminogenic, e.g., fluorescent or colorimetric, and luminogenic assays, e.g., in the same well, to detect the amount (e.g., activity) or presence in a sample of one or more moieties, including cofactors for enzymatic reactions such as ATP, proteins (peptides or polypeptides) that bind to and/or alter the conformation of a molecule, e.g., proteins that modify or cleave a peptide or polypeptide substrate, or a molecule which is bound by and/or altered by a protein. As used herein, a "luminogenic assay" includes a reaction in which a first molecule, e.g., a peptide or polypeptide substrate for a first enzyme, the product of a reaction between the first molecule and an appropriate (first) protein, and/or a product of a reaction between a different protein and the product of the first reaction, is luminogenic. Thus, a luminogenic assay may directly or indirectly detect, e.g., measure, the amount or presence of a cofactor for a reaction, a molecule which is bound by and/or altered by a protein, or the protein. For instance, in one embodiment, a beetle luciferase and an appropriate luciferin substrate may be employed in a luminogenic assay to detect ATP concentration, while in another embodiment a substrate for a luciferase, which is modified to contain a protease recognition site (modified, for example, via a covalent bond), may be employed in a luminogenic assay to detect the protease, i.e., when luciferase is present. Luminogenic assays include chemiluminescent and bioluminescent assays including but not limited to those which employ or detect luciferase, β-galactosidase, β-glucuronidase, β-lactamase, a protease, alkaline phosphatase, or peroxidase, and suitable corresponding substrates, e.g., modified forms of luciferin, coelenterazine, luminol, peptides or polypeptides, dioxetanes, dioxetanones, and related acridinium esters. As used herein, a "luminogenic assay reagent" includes a substrate, as well as a cofactor(s) or other molecule(s) such as a protein, e.g., an enzyme, for a luminogenic reaction. In one embodiment, the luminogenic assay reagent may be Z-DEVD-aminoluciferin (DEVD corresponds to SEQ ID NO:9), Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22), Z-LEHD-

aminoluciferin (LEHD corresponds to SEQ ID NO:11), or may be other substrates, e.g., peptide or polypeptide substrates, linked to aminoluciferin, dihydroluciferin, luciferin 6' methylether, or luciferin 6' chloroethylether. A luminogenic assay is one in which a luminogenic reaction yields at least 1%, e.g., at least 10%, more light than a corresponding nonluminogenic assay.

Please amend the paragraph starting on page 5, line 11 as follows:

A "nonluminogenic assay" includes a reaction in which a first molecule, e.g., a protein (a peptide or polypeptide), a (first) product of a reaction between the first molecule and a suitable (first) protein (peptide or polypeptide), or a product of a reaction between a different protein and the first product is/are not luminogenic but may be otherwise detectable, e.g., the substrate and/or product(s) are detected using a fluorescent or colorimetric assay, which directly or indirectly measures the amount or presence of a cofactor for the reaction, the molecule or the protein which interacts with the molecule. For instance, a substrate for an enzyme may be modified to contain a fluorophore that emits light of a certain wavelength only after the enzyme reacts with the substrate and the fluorophere is contacted with light of a certain wavelength or range of wavelengths, e.g., $(Z\text{-}DEVD)_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) is a substrate for a caspase, and cleavage of that substrate by the caspase may be monitored via fluorescence of rhodamine-110. As used herein, a "fluorogenic assay reagent" includes a substrate, as well as a cofactor(s) or other molecule(s), e.g., a protein, for a fluorogenic reaction. A nonluminogenic assay is one in which a nonluminogenic reaction yields less than about 10%, e.g., less than about 1% or less, the luminescent signal of a corresponding luminogenic assay.

Please amend the paragraph starting on page 6, line 8 as follows:

As described herein, the amount or presence of more than one protease in a sample was detected using at least two different substrates, one which had a luminescent readout and one or more of which had a fluorescent readout. For example, detection of a low abundance cellular protease was achieved using a more sensitive luminescent approach, e.g., detection of caspase-8 with the substrate Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22), followed by a detection of another protease using another substrate, for instance, caspase-3 with $(Z\text{-}DEVD)_2$-

rhodamine-110 (DEVD corresponds to SEQ ID NO:9). This assay thus combines the strengths of both a fluorogenic reagent and the sensitivity of a luciferase-mediated luminescent reaction. Moreover, surprisingly, the presence of a luciferin, a molecule which has fluorescent properties and is often present in relatively large quantities in luminescent assays, did not result in significant interference in combined fluorescent/luminescent assays. Further, surprisingly, two caspases and a luciferase were detected in the same reaction mix, a mix which included a caspase-8 substrate (Z-LETD-aminoluciferin; LETD corresponds to SEQ ID NO:22) and two caspase-3 substrates, i.e., $(Z\text{-DEVD})_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) and Ac-DEVD-AMC (DEVD corresponds to SEQ ID NO:9). The present invention thus provides more flexibility in molecules to be employed in multiplex assays, e.g., substrates for a luminogenic assay in combination with substrates for a fluorogenic assay. Moreover, if two enzyme-mediated reactions have compatible reagent conditions, the assay can be a one-step assay.

Please amend the paragraph starting on page 14, line 12 as follows:

Figures 1A-B. Multiplex assay measuring the enzyme activities of caspase-3 and caspase-8 in the presence of both luminogenic and fluorogenic assay reagents. A) Relative light units (RLU) versus time. DEVD corresponds to SEQ ID NO:9. B) Relative fluorescence units (RFU) over time. DEVD corresponds to SEQ ID NO:9; LETD corresponds to SEQ ID NO:22.

Please amend the paragraph starting on page 14, line 21 as follows:

Figures 4A-D. Multiplex assay measuring a protease (caspase-3) and a non-protease (β-galactosidase) enzyme. A) and C), RLU at 1/2 hour and 18 hours, respectively. DEVD corresponds to SEQ ID NO:9. B) and D) RFU at 2 hours and 18 hours, respectively. DEVD corresponds to SEQ ID NO:9.

Please amend the paragraph starting on page 14, line 24 as follows:

Figures 5A-C. Excitation and emission spectra of luciferin (A), aminoluciferin (B) and Z-LETD-aminoluciferin (C; LETD corresponds to SEQ ID NO:22).

Please amend the paragraph starting on page 14, line 26 as follows:

Figure 6. Signal to background ratios in three channels; rhodamine-110, AMC and luminescence, in a caspase-3 assay. DEVD corresponds to SEQ ID NO:9.

Please amend the paragraph starting on page 21, line 2, directly beneath Table 1, as follows:

X is one or more amino acids; W/LEHD-X corresponds to SEQ ID NO:1; DEXD-X corresponds to SEQ ID NO:2; L/VEXD-X corresponds to SEQ ID NO:3; IEGR-X corresponds to SEQ ID NO:4; E(N)XYXQ-S/G corresponds to SEQ ID NO:5; PRNK-X corresponds to SEQ ID NO:6; EISEVK/NM/L-DAEFRHD corresponds to SEQ ID NO:7; SEVNL-DAEFR corresponds to SEQ ID NO:8.

Please amend the paragraph starting on page 27, line 13 as follows:

Caspase-Glo™ 8 Reagent (Caspase-Glo™ 8 Assay System, Promega, Corp.) was evaluated for its ability to allow multiplexing of homogeneous luminogenic caspase-8 and nonluminogenic caspase-3 enzyme assays. Caspase-Glo™ 8 Reagent is comprised of Caspase-Glo™ 8 Buffer and the luminogenic substrate Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22). For the luminogenic assays in Figure 1A, either Caspase-Glo™ 8 Reagent (diamonds) or Caspase-Glo™ 8 Reagent also containing 50μM of the fluorogenic substrate for caspase-3, $(Z\text{-DEVD})_2$-rhodamine-110 (squares; DEVD corresponds to SEQ ID NO:9), was used to demonstrate the feasibility of a multiplexed luminogenic and nonluminogenic assay. For the fluorogenic assay in Figure 1B, Caspase-Glo™ 8 Buffer containing either 50 μM $(Z\text{-DEVD})_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) and 10 mM DTT (diamonds) or 50 μM $(Z\text{-DEVD})_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) and Z-LETD-aminoluciferin (squares; LETD corresponds to SEQ ID NO:22) were used.

Please amend the paragraph starting on page 27, line 24 as follows:

Dilutions of caspase-8 enzyme, caspase-3 enzyme, and combined caspase-8 and caspase-3 enzymes (Biomol Research Laboratories) were prepared in RPMI 1640 (Sigma Corporation) to a final concentration of 100 units/ml. 100 µl of caspase-8 dilutions, a mixture of caspase-8 and caspase-3 dilutions, or caspase-3 dilutions, were added to separate wells of a 96-well plate. 100 µl of Caspase-Glo™ 8 Reagent with or without 50 µM $(Z\text{-}DEVD)_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9, Figure 1A), or 100 µl of Caspase-Glo™ 8 Buffer supplemented with $(Z\text{-}DEVD)_2$-rhodamine 110 (DEVD corresponds to SEQ ID NO:9) and DTT with or without Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22; Figure 1B) were added to reach a final volume of 200 µl/well. The reaction plate was incubated at room temperature for at least ten minutes on a plate shaker.

Please amend the paragraph starting on page 28, line 8 as follows:

The simultaneous measurement of fluorescence and luminescence for two protease enzymes in a single well is shown in Figure 1. As seen in Figure 1A, the presence of caspase-3 and its fluorogenic substrate, $(Z\text{-}DEVD)_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9), in a luminogenic assay for caspase-8 (squares) does not greatly alter the luminescent reaction. Similarly, as seen in Figure 1B, the presence of caspase-8 and its luminogenic substrate Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22) in a fluorogenic assay for caspase-3 (squares) does not impact the fluorogenic assay for caspase-3.

Please amend the paragraph starting on page 28, line 16 as follows:

Various concentrations of luminogenic and fluorogenic reagents, including caspase enzymes and substrates thereof, and buffer components were combined to establish each constituent's contribution to fluorescence and/or luminescence. The fluorogenic substrate $(Z\text{-}DEVD)_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) reports caspase-3 activity in the rhodamine channel ($485_{EX}/520_{EM}$) and the fluorogenic substrate Ac-DEVD-AMC (DEVD corresponds to SEQ ID NO:9) reports the caspase-3 activity in the AMC channel ($360_{EX}/460_{EM}$), while the substrate Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22) reports caspase-8 activity during luminescence measurement. Table III describes the amount of each

component (μl) for twelve different reaction conditions resulting in a total volume of around 500 μl of master mix, or 100 μl of master mix/reaction (n=4) for each reaction condition. For the 'caspase added' row, the number in this row defines the type of caspase added in overabundance and does not describe a volume.

Please amend the paragraph starting on page 29, line 4, directly below Table III as follows:

DEVD corresponds to SEQ ID NO:9.

Please amend the paragraph starting on page 31, line 11 as follows:

For Figures 2A, B, and C, all carats represent where either fluorescence or luminescence indicating enzyme activity was expected. Figure 2A shows the signal for AMC fluorescence in each reaction. Fluorescence above background was only present where the appropriate substrate/enzyme combination of Ac-DEVD-AMC (DEVD corresponds to SEQ ID NO:9) and caspase-3 was present (reaction conditions 3 and 6). Figure 2B shows the signal for rhodamine-110 fluorescence in each reaction. Fluorescence above background was present where the substrate/enzyme combination of $(Z\text{-DEVD})_2$-rhodamine-110/caspase-3 (DEVD corresponds to SEQ ID NO:9) was present (reaction conditions 2, 4, 10 and 12), except when a caspase-3 inhibitor was present (reaction condition 5). For luminescence signal above background (Figure 2C), those reactions with the appropriate substrate/enzyme combination of Z-LETD-aminoluciferin/caspase-8 (LETD corresponds to SEQ ID NO:22) showed signal above background (reaction conditions 1, 4, 6, 7, 9, and 10), except those reaction conditions where a caspase inhibitor was present (reaction conditions 5, 8, and 11). The data thus demonstrate that there was negligible contribution of reaction components to background fluorescence and luminescence measurements under these conditions.

Please amend the paragraph starting on page 31, line 28 as follows:

Dilutions of detectable levels of caspase-8 (150 units/ml, Biomol Research Laboratories), caspase-3 (Pharmingen Corp.), trypsin (Sigma Corp.), and a combination of all three enzymes, were prepared in Dulbecco's phosphate buffered saline (Sigma Corp.). 100 µl of each enzyme dilution were added to the wells of a 96-well plate and 100µl of each substrate, either singly or in combination as appropriate, were added to the corresponding wells: substrate $(Z\text{-}DEVD)_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) for caspase-3, substrate Z-PRNK-AMC (PRNK corresponds to SEQ ID NO:24) for trypsin (as described in U.S. patent application Serial No. 09/955,639 as a substrate for beta-tryptases but with a recognized lesser utility for trypsin, incorporated herein in its entirety), and substrate Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22) for caspase-8. When Caspase-Glo™ 8 Buffer was employed with a substrate for a fluorogenic assay, 10mM DTT was included. Plates were incubated for at least ten minutes at room temperature on a plate shaker.

Please amend the paragraph starting on page 32, line 24 as follows:

Reagents were prepared by reconstituting Beta-Glo® lyophilized substrate with Beta-Glo® Buffer (Beta-Glo® Assay System, Promega Corp.), or adding $(Z\text{-}DEVD)_2$-rhodamine-110 (50 µM; (DEVD corresponds to SEQ ID NO:9) to Beta-Glo® Buffer, or reconstituting Beta-Glo® lyophilized substrate with Beta-Glo® Buffer and adding $(Z\text{-}DEVD)_2$-rhodamine-110 (50 µM; (DEVD corresponds to SEQ ID NO:9). Caspase-3 (2 µl/ml, Pharmingen Corp), or β-galactosidase (0.1 µl/ml), or caspase-3 and β-galactosidase, were diluted in RPMI 1640 and 100 µl were added to wells of a 96-well white plate. 100 µl of the appropriate reagent were added to wells of a 96-well plate and the plates were incubated at room temperature. Luminescence was measured using a DYNEX Laboratories MLX™ plate luminometer at 30 minutes. Fluorescence was measured 2 hours post incubation on a CytoFluor II Fluorescent plate reader with a filter set of $485_{EX}/530_{EM}$. All measurements were repeated at 18 hours with different gain settings on the CytoFluor II fluorometer to compensate for increased fluorescence.

Please amend the paragraph starting on page 33, line 16 as follows:

Luciferin, aminoluciferin, and Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22), were diluted to approximately 2 μM in a buffer containing 0.1M Tris pH 7.3, 2 mM EDTA, and 10 mM $MgSO_4$. Samples were scanned on a SPEX Fluorolog-2 spectrometer with 1.25 mm excitation and emission slit filter present, at 1 nm wavelength interval and 0.2 second integration time. All scans were performed using a quartz cuvette.

Please amend the paragraph starting on page 33, line 22 as follows:

For luciferin and aminoluciferin, excitation was at 325 nm and emission was captured from 375 to 750 nm, and excitation was captured at 280-550 nm with emission measured at 600 nm (Figures 5A and 5B). For Z-LETD-aminoluciferin (LETD corresponds to SEQ ID NO:22), excitation was at 325 nm and emission was captured between 375-750 nm, and excitation was captured at 280-500 nm with emission measured at 525 nm (Figure 5C). Interestingly, when a peptide was conjugated to aminoluciferin (Figure 5C), the emission peak of the conjugate was blue shifted to shorter wavelengths. This was unexpected and therefore allows for dual luminscence/fluorescent measurements, particularly when using a fluorophore that emits in the same wavelength range as aminoluciferin emits.

Please amend the paragraph starting on page 34, line 5 as follows:

Caspase-Glo™ 3/7 Reagent (Caspase-Glo™ 3/7 Assay, Promega, Corp.) which contains Z-DEVD-aminoluciferin (DEVD corresponds to SEQ ID NO:9) was combined with $(Z\text{-DEVD})_2$-rhodamine-110 (DEVD corresponds to SEQ ID NO:9) or Ac-DEVD-AMC (DEVD corresponds to SEQ ID NO:9) in the presence of caspase-3 with either a caspase-3 inhibitor (Ac-DEVD-CHO, 10 μM; DEVD corresponds to SEQ ID NO:9) or with a luciferase inhibitor (Resveratol, 5 μM). The luminescent signal from caspase-3 cleavage of Z-DEVD-aminoluciferin (DEVD corresponds to SEQ ID NO:9) was read at 30 minutes, while the fluorescent signals from caspase-3 cleavage activity were read at 2 hours using the appropriate AMC or rhodamine 110 filter sets.

Please amend the paragraph starting on page 37, line 3 as follows:

The following detection reagents were prepared: 1) PKA reagent-a 1X reaction buffer was prepared which contains 100 mM Tris pH 7.3, 100 mM $MgCl_2$, 1:1000 dilution of a PKA rhodamine-110 substrate (ProFluor™ PKA Assay, Promega Corporation, Technical Bulletin 315), and 400 μM ATP; 2) caspase-3 reagent-a 1X reaction buffer was prepared containing 100 mM Tris pH 7.3, 100 mM $MgCl_2$, 150 μg/ml recombinant thermostable luciferase, 80 μM Z-DEVD-aminoluciferin (Promega Corp; DEVD corresponds to SEQ ID NO:9), 400 μM ATP, 100 μM DTT (Promega Corp), 2.5 mM $CaCl_2$ (Fisher), 40 mM $MgSO_4$ (Fisher), and 0.2% Tergitol NP-9 (Sigma); 3) kinase/caspase-3 combined reagent-a 1X reaction buffer was prepared containing 100 mM Tris pH 7.3, 100 mM $MgCl_2$, 1:1000 dilution of a PKA rhodamine-110 substrate, 150 μg/ml recombinant thermostable luciferase, 80 μM Z-DEVD-aminoluciferin (DEVD corresponds to SEQ ID NO:9), 400 μM ATP, 100 μM DTT, 2.5 mM $CaCl_2$, 40 mM $MgSO_4$, and 0.2% Tergitol NP-9; 4) protein kinase stop reagent- a 1X stop reagent was prepared containing 100 mM Tris pH 7.3, 100 mM $MgCl_2$, 1:50 dilution of protease reagent (ProFluor™ PKA Assay), 30 μM staurosporine (BIOMOL Laboratories).

Please amend the paragraph starting on page 38, line 14 as follows:

The following detection reagents were prepared: 1) EnduRen™ (Promega Corp.), a cell permeant modified coelenterazine substrate for *Renilla* luciferase, was diluted to 600 μM into F-12 tissue culture medium supplemented with 10% fetal bovine serum and 500 μg/ml G-418 sulfate; 2) caspase-3 substrate: $(Z\text{-}DEVD)_2$-rhodamine-110 (Promega Corp.; DEVD corresponds to SEQ ID NO:9) was diluted to 250 μM into F-12 tissue culture medium supplemented with 10% fetal bovine serum and 500 μg/ml G-418 sulfate; 3) luciferase/caspase-3 combined substrates:EnduRen™ (600 μM) and $(Z\text{-}DEVD)_2$-rhodamine-110 (250 μM; DEVD corresponds to SEQ ID NO:9) were diluted in F-12 tissue culture medium supplemented with 10% fetal bovine serum and 500 μg/ml G-418 sulfate.